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Glucose and the Metabolism of Adenosine 3′:5′-Cyclic Monophosphate in *Escherichia coli*

(growth/glucose ultilization/cAMP production/extracellular cAMP)

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ABSTRACT **Measurements of adenosine 3′:5′-cyclic monophosphate (cAMP) concentrations have been made in *Escherichia coli* under various conditions. Different strains of *E. coli* accumulate different extracellular concentrations of cAMP (0.2–4 μm) at stationary phase. Mutation at the RNA control locus does not affect the accumulation pattern. Growth of the bacteria in minimal-salts medium leads to a greater accumulation of cAMP than growth in nutrient broth. Partition studies show that essentially all of the cAMP that is accumulated is found in the medium rather than in the cells. Kinetic studies show that most of the cAMP is formed coincidentally with exhaustion of glucose from the medium. Growth on high concentrations of glucose leads to inhibition of cAMP formation. Other carbon sources cannot substitute for glucose in this inhibitory effect. Measurements of enzyme activities indicate that glucose suppression of cAMP formation cannot be accounted for by a decreased activity of adenylate cyclase or an increased activity of cAMP phosphodiesterase (EC 3.1.3.7).**

Several years ago Makman and Sutherland (1) reported the occurrence of adenosine 3′:5′-cyclic monophosphate (cAMP) in *Escherichia coli* and characterized some of the conditions under which its concentration varied inside the cells. In the intervening years, the importance of this nucleotide in induced enzyme synthesis has become apparent (2). The present study was undertaken to explore further the metabolism of cAMP in *E. coli*. We have extended the observations of Makman and Sutherland (1) by showing that during the growth of *E. coli* substantially more total nucleotide accumulates outside the cells than is found intracellularly. In exploring the effect of glucose on the pattern of cAMP accumulation, we found that glucose suppresses the formation of extracellular cAMP. A possible enzymatic basis for the effect of glucose inhibition of cAMP formation was investigated. The biological significance of the variation of extracellular concentrations of cAMP is not clear.

MATERIALS AND METHODS

Bacteria. E. coli CP 78 and 79, and strains 15 TAU (stringent and relaxed): kindly provided by Dr. Robert Lazzarini.

Protein kinase for the determination of cAMP was a generous gift of Dr. Alfred Gilman. Glucostat reagent was purchased from Worthington Co. Hydroxyurea was a product of Nutritional Biochemical Corp., chloramphenicol-succinate was from Parke-Davis, and rifamycin was donated by Dr. Robert Lazzarini. [α-^{32}P]ATP (8.2 Ci/mmol) was from New England Nuclear Corp.; [^{3}H]cAMP (16.3 Ci/mmol) and oxidized glutathione were from Schwarz BioResearch. Unlabeled cAMP was from Calbiochem. Snake venom (*Bothrops atrox*) was from Sigma.

Growth of Bacteria. Standard medium was the minimal-salts medium described by Vogel and Bonner (3), unless otherwise designated. Incubation was with shaking in a New Brunswick Gyrotory shaker. Measurements of absorbance at 650 nm were made in a Gilford model 300 micro-sample spectrophotometer.

cAMP Concentration. Samples for the determination of cAMP were initially treated in a boiling-water bath for 3 min. They were then cooled and cell debris was removed by centrifugation. The supernatant fractions were adjusted to pH 4 with HCl. Aliquots of these fractions were assayed for cAMP content by the protein-binding procedure described by Gilman (4). Since the assay depends on competition for binding of [^{3}H]cAMP to protein, it was essential to prove that the material assayed was actually cAMP and not a nonspecific inhibitor. This was done by showing that the binding-competition activity of a typical sample (*a*) fractionated on Dowex-50 as did authentic cAMP and (*b*) was destroyed by cAMP phosphodiesterase. Calculations of cAMP concentration were made with the use of a computer program written by Dr. Florence K. Millar of the National Cancer Institute.

Glucose concentration. Samples for determination of glucose in culture medium were prepared in the same manner as those for determination of cAMP concentration. In most instances, the supernatant fractions were analyzed for glucose with the Glucostat reagent obtained from the Worthington Biochemical Corp., by a modification of Worthington Method II.

A vial of Glucostat X 4 reagent and a vial of chromogen were dissolved in 0.05 M phosphate buffer (pH 7) to a volume of 200 ml. A sample for assay that contained from 0.01 to 0.05 mg of glucose in a volume of 1 ml was mixed with 1 ml of the Glucostat reagent. After 10 min of incubation at room temperature, enough 4 N HCl was added to bring the pH to 3. 5 min after the addition of acid, the absorbance of the sample at 400 nm was measured in the Gilford spectrophotometer.

In the experiment of Table 3, it was found that hydroxyurea interfered with the assay of glucose with the Glucostat reagent. Therefore, for this experiment, glucose was determined colorimetrically by the phenol–sulfuric acid method described by Ashwell (5).

Adenylate Cyclase Assay. The assay was derived from those described by Tao and Huberman (6) and Krishna *et al.* (7), except that a large pool of unlabeled cAMP was included to dilute out the competing effect of any cAMP phosphodiesterase present. The assay contained, in a total volume of 0.05 ml: Tris·HCl (pH 8.5), 2.5 μmol, $MgCl_2$, 1.0 μmol, [α-^{32}P]-

ATP, 15 nmol (about 10^6 cpm), cAMP, 2 nmol, and the enzyme to be assayed.

Incubation was at 34°C for 30 min. The incubation tubes were diluted with 0.5 ml H_2O containing 1.25 μmol of cAMP, and immersed in a boiling-water bath for 2 min. The sample was applied to a column (0.5 × 3.3 cm) of Dowex-50W ×2 200–400 mesh and washed through with 1 ml of H_2O. The next 4 ml of H_2O wash was collected in a test tube. This 4-ml fraction was carried through two cycles of barium–zinc precipitation, as described by Krishna *et al.* (7). The supernatant solution was poured into a scintillation counting vial and counted with 15 ml of Triton–toluene–liquifluor 6:12:1. Boiled-enzyme samples were used as controls.

cAMP Phosphodiesterase Assay. This assay was derived from that described by Brooker *et al.* (8) for the assay of cAMP. The assay contained, in a total volume of 0.2 ml: Tris·HCl (pH 8), 10 μmol, EDTA, 0.1 μmol, $MgCl_2$, 6 μmol, 5′-AMP, 5 nmol (about 4500 cpm of ^{14}C), [^3H]cAMP, 25 nmol (about 45,000 cpm), bovine-serum albumin, 0.1 mg, dithiothreitol, 0.25 μmol, snake venom, 0.1 mg, and the enzyme to be assayed. The added snake venom contained sufficient phosphatase activity to convert all the 5′-AMP formed to adenosine. The samples were incubated for 15 min at 30°C, then diluted with 0.5 ml of H_2O and applied to a column (0.5 × 4 cm) of Dowex 3×4 100–200 mesh. The next 2 ml of H_2O wash was collected in a scintillation counting vial. 15 ml of Triton–toluene–liquifluor counting fluid was added and the ^3H and ^{14}C content of the samples was determined in a Packard scintillation counter. The [^3H]adenosine formed due to the addition of enzyme was corrected for the amount of [^{14}C]adenosine recovered though the fractionation procedure.

Glutathione Reductase (EC 1.6.4.2). This was assayed by the method described by Racker (9). Incubation was at room temperature in a Gilford recording spectrophotometer with an automatic sample changer.

RESULTS

Since cAMP has been involved in the synthesis of mRNA for induced enzymes (2), it was of interest to determine whether cAMP concentrations correlated with lesions in RNA synthesis. Some isogenic pairs differing only in the region concerned with the regulation of RNA synthesis (the *RC* locus) were tested for cAMP concentrations. It can be seen (Table 1) that stationary cultures of *E. coli* CP-78 or CP-79 in nutrient broth accumulate comparable concentrations of cAMP. A similar picture is seen with an isogenic RNA-control pair in the *E. coli* 15 family. While the concentrations of cAMP formed by the *E. coli* 15 pair were only about 25% of that formed by the CP mutants, there was no significant difference between the stringent or relaxed-control mutants. Wild-type *E. coli* B cultured in nutrient broth accumulated a concentration of cAMP about intermediate between that of the 15 and CP strains. A test of the effect of culture conditions on the pattern of cAMP formation showed that stationary cells of *E. coli* B grown in minimal-salts medium with glucose as carbon source accumulated over five times more cAMP than did the same strain grown in nutrient broth.

TABLE 1. *Formation of cyclic AMP by different strains of E. coli*

Strain	Culture medium	A_{650}	cAMP (pmol/ml)
CP 78 (RC^{str})	Nutrient Broth	0.75	1240
CP 79 (RC^{rel})	Nutrient Broth	0.80	1450
15 TAU (RC^{str})	Nutrient Broth	0.97	400
15 TAU (RC^{rel})	Nutrient Broth	0.81	250
B	Nutrient Broth	0.98	700
B	Minimal + glucose	1.8	3880

Cultures were incubated for 2 days, with shaking, at 37°C, at which time there was no further increase in cell density. The absorbance at 650 nm was determined. A sample of the cell culture was assayed for cAMP as described under *Methods*.

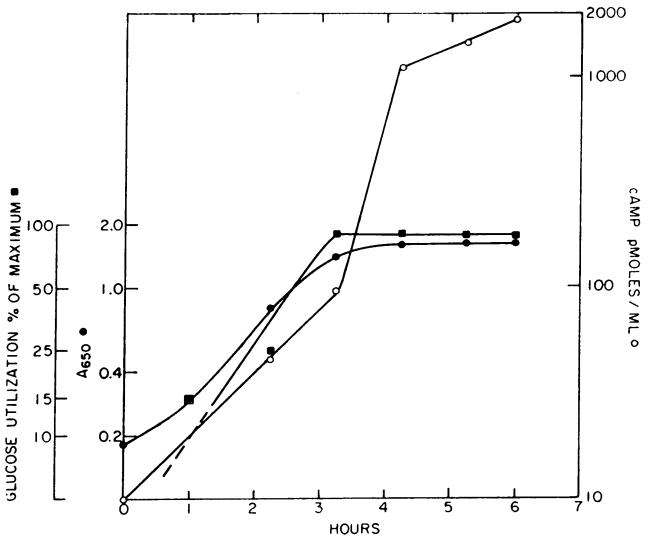

FIG. 1. Time course of growth, glucose utilization, and cAMP accumulation by *E. coli* B. An overnight culture of *E. coli* B was centrifuged and washed once, then resuspended to a density of about 0.2 at 650 nm in minimal-salts medium (3) supplemented with 0.3% glucose. The culture was shaken at 37°C and samples were removed periodically for the determination of cell density, glucose, and cAMP.

Since the data of Table 1 showed that the maximum accumulation of cAMP took place in minimal-salts medium, the parameters affecting the accumulation of cAMP were further explored under these conditions. Fig. 1 shows the rate of growth compared with the accumulation of cAMP and the utilization of glucose by a growing culture of *E. coli* B. It should be noted that the data is plotted on a semilogarithmic scale. The noteworthy point in the experiment shown is that at 3 hr the concentration of cAMP is only 5% of the maximal, although maximal growth of the culture has been attained. Shortly after maximum growth was attained, there was an abrupt rise in the concentration of cAMP in the culture—a change from about 100 pmol/ml to about 1000 pmol/ml over a period of 1 hr. It is not clear from this experiment whether the accumulation of cAMP required a cessation of growth or was due to some other factor. Fig. 1 also shows the rate of glucose utilization by the growing culture of *E. coli* B; the glucose has been depleted from the culture at the time at which there was a marked rise in cAMP concentration. The studies of Makman and Sutherland (1) have suggested that glucose affects the formation of intracellular cAMP by resting cells of *E. coli* B. The present data, which suggest a correlation between

TABLE 2. *Extracellular distribution of cAMP accumulated during growth of E. coli B*

Hours	A_{650}	cAMP (pmol/ml) Total	In medium
0	0.18	<10	<10
1	0.29	<10	<10
2.25	0.80	50	50
3.25	1.4	100	100
5.25	1.6	1450	1450

E. coli was grown as in Fig. 1. At the designated time intervals, duplicate samples (about 2 ml) of the culture were removed. One sample (for determination of cAMP in medium) was centrifuged to remove the cells and the supernatant was placed in a boiling-water bath for 3 min. The other sample was heated before removal of the cells. After they were heated, the samples were clarified by centrifugation.

glucose depletion and the increase in cAMP concentration agree with those data.

The studies of Makman and Sutherland (1), which indicated that the addition of glucose to a resting-cell suspension of *E. coli* B resulted in excretion of the preexisting pool of cAMP to the medium, suggested the study described in Table 2. The concentration of cAMP within the cells, as well as in the growth medium, was determined throughout the growth of the culture of *E. coli* B. Samples for analysis of cAMP were taken at points at which the total cAMP concentration was low, during the early part of the growth curve, as well as when the cAMP concentration was high, after stationary phase had been reached. The data show that at all points during the growth curve, the amount of cAMP in the total culture is the same as that in the medium outside of the cells. It should also be noted that the post-logarithmic concentration of cAMP in the medium measured here (1.45 μM) is about ten times *lower* than that reported by Makman and Sutherland (1) as the intracellular concentration of cAMP (10 μM) at the same point in the growth curve. However, since the volume occupied by the cells is less than 0.1% of the total culture volume, the fraction of the total cAMP concentration found intracellularly would be obscured in our studies.

To investigate whether the cessation of macromolecular synthesis characteristic of stationary growth is necessary for

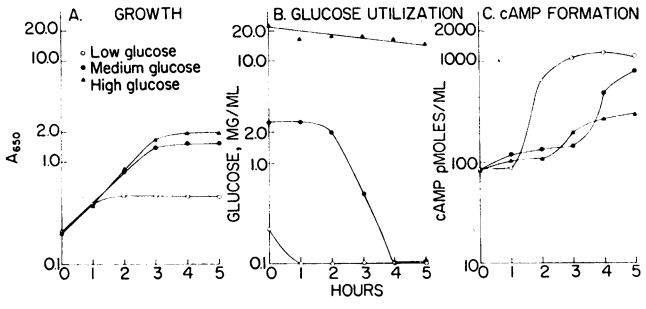

FIG. 2. Effect of glucose concentration on growth, glucose utilization, and cAMP accumulation by E. coli B. Three 100-ml aliquots of minimal-salts medium containing different concentrations of glucose (0.03, 0.3, or 3%) were inoculated with a washed suspension of *E. coli* B as in Fig. 1. The cells were incubated with shaking at 37°C. At the indicated periods, samples were removed for determination of cell density, glucose, and cAMP.

cAMP formation, the experiment described in Table 3 was performed. Hydroxyurea (an inhibitor of DNA synthesis), rifamycin (an inhibitor of RNA synthesis), and chloramphenicol (an inhibitor of protein synthesis) were tested for their effects on growth, glucose utilization, and cAMP formation. In a normal culture, after 4 hr of growth, about 3000 pmol/ml of cAMP was found in the medium, while all three metabolic inhibitors completely blocked the formation of cAMP. Coincident with the inhibition of cAMP formation, all three inhibitors depressed the extent of growth, and also substantially inhibited the utilization of glucose, by the cultures. It cannot be determined from this experiment whether the factors critical for the maximum formation of cAMP are the environmental conditions characteristic of high cell density, such as the accumulation of waste products or the reduction in oxygen tension, or whether the critical factor is the complete utilization of glucose. The experiment detailed in Fig. 2 was designed to answer this question. The effect of glucose concentration in the culture medium on growth, glucose utilization, and cAMP formation was determined. Separate cultures of *E. coli* B were grown in medium containing a concentration of glucose that was sufficient to support only about 25% of maximum growth (low glucose, 0.03%); a concentration 10 times higher than that, sufficient to support maximum growth (medium glucose, 0.3%); and a high concentration of glucose (high glucose, 3.0%). The growth curves at these glucose concentrations are shown in Panel *A*. The curves for glucose utilization are shown in Panel *B*. In the low-glucose culture, there was a rapid disappearance of the sugar from the medium, more or less coincident with the cessation of growth. Similarly in the medium-glucose culture, growth was maximal at about 3 hr and glucose was exhausted 3- to 4-hr after growth started. A contrasting situation can be seen in the high-glucose culture. The growth curve was similar in both the high- and medium-glucose cultures, but the glucose utilization curves were quite different. In the case of the high-glucose culture, substantial concentrations of glucose remained in the culture long after the complete cessation of growth. The pertinent data for cAMP formation can be seen in Panel *C*, which shows that in the low-glucose culture cAMP was formed coincident with the cessation of growth, at a time that the glucose had been depleted from the medium. This result can

TABLE 3. *Effect of inhibitors on growth, glucose utilization, and cAMP formation*

Hours	Inhibitor added	Growth (A_{650})	Glucose remaining, (mg/ml)	cAMP (pmol/ml)
0	None	0.18	2.8	80
4	None	1.6	0.05	3290
4	Hydroxyurea	0.73	1.8	120
4	Chloramphenicol	1.07	1.8	180
4	Rifamycin	0.25	2.2	60

An overnight culture of *E. coli* B was centrifuged and resuspended in minimal-salts medium + 0.3% glucose. This suspension was used to inoculate a fresh culture to an initial absorbance value of 0.2. The cell suspension was incubated with shaking at 37°C. At 1 hr, inhibitors were added to aliquots of the cultures and shaking was continued. The final concentrations of inhibitors were as follows: 0.1 M hydroxyurea, 0.1 mg/ml rifamycin, 0.1 mg/ml chloramphenicol.

be contrasted with that shown in Table 3, where growth was inhibited by metabolic inhibitors and there was no cAMP formation. It can, therefore, be concluded that maximum cAMP formation *does not* require full growth of a culture, but is probably dependent on disappearance of glucose from the medium. The pattern of cAMP formation in the medium-glucose culture is comparable to that shown in Fig. 1. Again, the maximal accumulation of cAMP is coincident with the cessation of growth and the complete utilization of glucose from the medium. While both the medium- and the high-glucose cultures gave comparable amounts of growth, the concentration of cAMP formed in the high-glucose culture was substantially lower than that in the medium-glucose culture (800 vs. 300 pmol/ml). This difference becomes even more pronounced at 24 hr (Table 4). While this difference could not be attributed to a difference between the amount of growth, it seemed to reflect the incomplete utilization of glucose in the high-glucose culture. The tentative conclusion from these studies was that a high concentration of glucose in the medium leads to a substantial suppression of the formation of cAMP by cultures of *E. coli* B.

The specificity of this suppressive effect of high concentrations of glucose on the formation of cAMP was investigated by growth of cultures of bacteria on different concentrations of various carbon sources. As shown in Table 4, all the concentrations of the various carbon sources used gave comparable amounts of growth after a 24-hr incubation. The measurements of cAMP concentration, however, showed that when the glucose concentration was changed from 0.3 to 1%, there was a 95% inhibition of cAMP formation. A similar study with glycerol as the carbon source showed a much less severe inhibition of cAMP formation by higher concentrations of glycerol in the medium. At 1% glycerol, there was only 37% inhibition; inhibition increased to about 50% at the highest concentration (3%). With succinate as the carbon source, there was no difference in the accumulation of cAMP over a range from 1 to 5% succinate. This study suggests that the capacity to supress formation of cAMP is specific for glucose.

TABLE 4. *Effect of carbon source on growth and cAMP formation in E. coli B*

Carbon source	Concentration (%)	Growth (A_{650})	cAMP (pmol/ml)
Glucose	0.3	1.71	3980
Glucose	1	1.60	150
Glucose	2	1.54	140
Glucose	3	1.61	150
Glycerol	0.3	1.93	890
Glycerol	1	1.66	560
Glycerol	2	1.54	530
Glycerol	3	1.53	470
Succinate	1	1.26	1570
Succinate	2	1.34	1500
Succinate	3	1.28	1740
Succinate	5	1.05	1470

An overnight culture of *E. coli* B was centrifuged and resuspended in minimal medium to a cell density of 0.1 A_{650}. The carbon sources were added, after which the cell suspensions were incubated with shaking at 37°C for a further 24 hr.

TABLE 5. *Enzyme activities in extracts of E. coli B grown in different concentrations of glucose*

Glucose in medium (%)	Growth stage	Adenylate cyclase	cAMP phospho-diesterase	Glutathione reductase
		(nmol/15 min per mg of protein)		
0.03	Logarithmic	0.41	81	700
0.3	Logarithmic	0.37	104	621
3	Logarithmic	0.82	82	630
0.03	Post-logarithmic	0.29	74	410
0.3	Post-logarithmic	0.12	88	260
3.0	Post-logarithmic	0.06	17	290

Cultures of *E. coli* B were grown in minimal-salts medium containing the designated concentrations of glucose. The cells were harvested by centrifugation and resuspended in 0.01 M Tris·HCl (pH 7.5) at a concentration of about 0.15 g wet weight/ml. The cell suspension was sonicated in a test tube at peak power in a Raytheon Sonicator (10 Kc) for 30 min with cooling. Adenylate cyclase and cAMP phosphodiesterase activities were determined directly on the sonicates. Glutathione reductase activity was determined on supernatant fractions after centrifugation at 10,000 × *g* for 10 min.

It is also interesting to note from Table 4 that the maximum concentrations of cAMP formed varied widely with the carbon source.

In an attempt to discover the mechanism for the glucose suppression of cAMP formation, enzyme activities in cell-free extract were determined (Table 5). A mechanism consistent with our data would be that the enzyme that leads to the formation of cAMP, adenylate cyclase, should be present in low amounts or absent in logarithmic-phase cells, and its synthesis should be inhibited by glucose. Such a theory assumes that depletion of glucose leads to the induction of adenylate cyclase. To test this hypothesis, extracts were made of cells that had been grown with various concentrations of glucose. Extracts of cells harvested at the logarithmic stage of growth (A_{650} about 0.4), contained substantial amounts of adenylate cyclase, irrespective of the glucose concentration in the medium. If anything, the high-glucose culture shows a somewhat higher specific activity of the enzyme. When extracts are made from post-logarithmic cells (i.e., cells that have remained overnight on the shaker), the high-glucose cultures show significantly lower specific activities of adenylate cyclase than do the low-glucose cultures. It is not likely, however, that these activity differences can explain the inhibition of cAMP formation by high glucose cultures, since the accumulation of cAMP is already complete before 8–10 hr, at which point the cAMP concentration remains constant. Thus, there is no support for the theory that glucose leads to an inhibition of the induction of adenylate cyclase and that a deficit in the activity of this enzyme accounts for the lack of formation of cAMP in the presence of glucose.

A second possible theory to explain the suppression of cAMP formation by glucose is that the enzyme that leads to the degradation of cAMP, cAMP phosphodiesterase, is present in higher concentrations in cells grown in high-glucose medium. The data of Table 5 show that extracts of logarithmic-phase cells have comparable specific activaties of cAMP phospho-

diesterase (EC 3.1.3.7), irrespective of the glucose content in the culture medium. When extracts were made from post-logarithmic cells, the cAMP phosphodiesterase activity was not higher, but rather was lower, in the high-glucose culture. There was about 20% as much cAMP phosphodiesterase, as well as adenylate cyclase, in the high-glucose extracts as there was in the low-glucose extracts.

It has recently been suggested (10) that the expression of cAMP phosphodiesterase activity in *E. coli* requires glutathione reductase. For this reason, glutathione reductase activities were also tested in high- and low-glucose extracts. No substantial difference that was dependent on glucose concentration was observed for this activity, either. The explanation for the generally lower specific activities of all the enzymes in post-logarithmic extracts of high-glucose cultures is not clear. It may reflect an imbalance in protein synthesis or degradation in resting-cell cultures that contain a residual energy source. Nonetheless, an explanation of the glucose effect on cAMP accumulation based on changes in the activities of the synthetic or degradative enzymes was not supported by our data.

DISCUSSION

The studies reported here complement those of Makman and Sutherland (1). It is, however, useful to compare their data with ours. Most of their measurements of cAMP were made on cells after removal of the culture medium. A typical value for cAMP concentration was about 20,000 pmol/g wet weight of cells, the usual yield of cells from a liter of medium. On the other hand, our measurements of cAMP in the total culture show that the medium derived from that amount of cells contains about 200 times as much cAMP as they reported. This indicates that at all stages of growth, about 99% of the cAMP produced by *E. coli* is excreted into the culture medium. Data were also presented (1) that showed that cells grown in medium containing a high concentration of glucose accumulate less cAMP inside the cells after suspension of the cells in glucose-free buffer than do cells previously cultured in low-glucose medium. This observation is consistent with our observations that a medium containing a high-glucose concentration inhibits cAMP formation in the total culture. Their studies of the kinetics of cAMP formation in cells during growth showed an abrupt rise in the cellular cAMP concentration coincident with the complete utilization of glucose, followed by a rapid fall in cAMP concentration in cells. In contrast, our studies of the total cAMP concentration also show an abrupt rise in cAMP concentration coincident with the complete utilization of glucose. However, we see no fall in the cAMP concentration in the medium; the maximal concentration accumulated does not decrease even after several days. Their study also showed that incubation of resting cells with glucose led to release of cAMP into the medium; in contrast, our data indicate that maximal formation of cAMP takes place when glucose is depleted and the cyclic nucleotide is, nevertheless, found in the medium. We conclude therefore that if the synthesis of cAMP depends on the absence of glucose, the excretion must be dependent on some factor other than the presence of glucose.

On the basis of kinetic studies and an experiment with chloramphenicol present to inhibit protein synthesis, Makman and Sutherland (1) concluded that the increase in cAMP concentration was not dependent on induction of a new enzyme(s). Our measurements of the activities of adenylate cyclase, cAMP phosphodiesterase, and glutathione reductase also indicate that the variations in cAMP concentration we observed are not dependent on *de novo* synthesis of enzyme(s). The possibility that glucose or its metabolites regulate the activity of adenylate cyclase (6) is not excluded. We have no adequate biochemical explanation for either the kinetics of accumulation of cAMP that we observed, or for the inhibition of cAMP formation by glucose. It should be pointed out that in these studies, as well as those of Makman and Sutherland (1), measurements were made of the accumulation of cAMP and not of the rate of formation of the nucleotide. It seemed possible that the inability of logarithmic cultures or cultures grown in the presence of a high concentration of glucose to accumulate cAMP was due to the degradation of the nucleotide by intracellular cAMP phosphodiesterase. This suggestion, however, would not be consistent with the conclusion of Makman and Sutherland (1) that glucose leads to the excretion of cAMP. In order to characterize the phenomenon further, it will be necessary to determine the rates of synthesis and the partition of cAMP between cells and medium during growth under various conditions.

No role has yet been assigned to the variation in extracellular concentrations of cAMP in *E. coli*. Some slime molds secrete cAMP (11). Studies from Bonner's laboratory (12) have shown that acrasin, the hormone necessary for the aggregation step in the morphogenesis of slime molds, is cAMP.

We are indebted to Dr. Alfred Gilman for his introduction to, and advice on, the cAMP assay. Dr. Florence K. Millar of the National Cancer Institute lent her expertise to the development of a computer program for calculating data generated from cAMP assays. Dr. Robert Lazzarini provided several useful bacterial strains and Mrs. Helmi Carpenter assisted in the preparation of the manuscript.

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